



10/03/1366

INVESTOR IN PEOPLE

The Patent Office
Concept House
Cardiff Road
Newport
South Wales

NPI0 8QQ

10 AUG 2000

GB00/02230

I, the undersigned, being an officer duly authorised in accordance with Section 74(1) and (4) of the Deregulation & Contracting Out Act 1994, to sign and issue certificates on behalf of the Comptroller-General, hereby certify that annexed hereto is a true copy of the documents as originally filed in connection with the patent application identified therein.

In accordance with the Patents (Companies Re-registration) Rules 1982, if a company named in this certificate and any accompanying documents has re-registered under the Companies Act 1980 with the same name as that with which it was registered immediately before re-registration save for the substitution as, or inclusion as, the last part of the name of the words "public limited company" or their equivalents in Welsh, references to the name of the company in this certificate and any accompanying documents shall be treated as references to the name with which it is so re-registered.

In accordance with the rules, the words "public limited company" may be replaced by p.l.c., plc, P.L.C. or PLC.

Re-registration under the Companies Act does not constitute a new legal entity but merely subjects the company to certain additional company law rules.

Signed

Dated 28 JUL 2000

**PRIORITY
DOCUMENT**

SUBMITTED OR TRANSMITTED IN
COMPLIANCE WITH RULE 17 (a) OR (b)

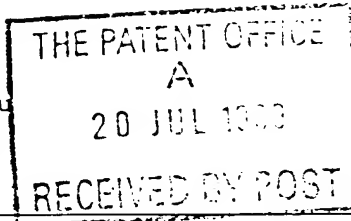


16850.2

2E

Request for grant of a patent

(See the notes on the back of this form. You can also get an explanatory leaflet from the Patent Office to help you fill in this form)



The Patent Office

Cardiff Road
Newport
Gwent NP9 1RH

1. Your reference

23359

2. Patent application number

(The Patent Office will fill in this part)

3. Full name, address and postcode of the or of each applicant (underline all surnames)

University of Wales, Bangor, College Road, Bangor, Gwynedd L57 2DG

Patents ADP number (if you know it)

If the applicant is a corporate body, give the country/state of its incorporation

United Kingdom

4. Title of the invention

DIELECTROPHORETIC APPARATUS & METHOD

5. Name of your agent (if you have one)

GALLAFENT & CO

"Address for service" in the United Kingdom to which all correspondence should be sent (including the postcode)

9 STAPLE INN
LONDON WC1V 7QH

Patents ADP number (if you know it)

0000729001 ✓

If you are declaring priority from one or more earlier patent applications give the country and the date of filing of the or of each of these earlier applications and (if you know it) the or each application number

Country

Priority application number
(if you know it)

Date of filing
(day/month/year)

7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application

Number of earlier application

Date of filing
(day/month/year)

8. Is a statement of inventorship and of right to grant of a patent required in support of this request? (Answer 'Yes' if:

- a) any applicant named in part 3 is not an inventor, or
 - b) there is an inventor who is not named as an applicant, or
 - c) any named applicant is a corporate body.
- (See note (d))

Yes

9. Enter the number of sheets for any of the following items you are filing with this form. Do not count copies of the same document

Continuation sheets of this form

| | | |
|-------------|----|----|
| Description | 23 | / |
| Claim(s) | 4 | / |
| Abstract | 1 | / |
| Drawings | 10 | 10 |

10. If you are also filing any of the following, state how many against each item.

Priority documents

Translation of priority documents

Statement of inventorship and right to grant of a patent (Patents Form 7/77)

Request for preliminary examination and search (Patents Form 9/77) 1 /

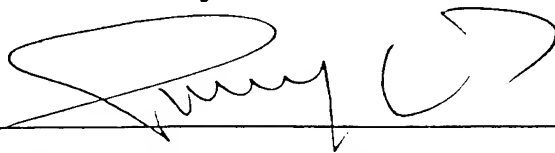
Request for substantive examination and search (Patents Form 10/77)

Any other documents
(Please specify)

11. I/We request the grant of a patent on the basis of this application.

Signature

Date



19 July 1999

12. Name and daytime telephone number of person to contact in the United Kingdom

GALLAFENT & CO
0171 242 3094

DIELECTROPHORETIC APPARATUS & METHOD

This invention relates to an apparatus and method of using the technique of dielectrophoresis, and relates particularly to an arrangement for concentrating or diluting or transporting or separating or detecting or characterising particles.

The technique of dielectrophoresis (DEP) is described in the book "Nanotechnology in Medicine and the Biosciences", Ed RRH Combs and D W Robinson, published by Gordon & Breach, Amsterdam, chapter 11 by Ronald Pethig, especially pages 88 to 93. Dielectrophoresis is the movement of particles in non-uniform electric fields. Unlike electrophoresis, charges on the particle itself are not necessary for the effect to occur and AC rather than DC fields are employed.

When an electric field is applied to a system consisting of particles suspended in a liquid medium, a dipole moment is usually induced in each particle as a result of electrical polarisations forming at the interfaces that define their structure. If the field is non-uniform, the particles experience a translational force, known as a dielectrophoretic force, of magnitude and

polarity dependent on the electrical properties of the particles and their surrounding medium. This force is also a function of the magnitude and frequency of the applied electric field.

5

One application of the technique of DEP is described in WO 98/04355, British Technology Group, in which a

particle-containing liquid is caused to flow over a comb-like array of electrodes to which signals at
10 different frequencies are applied; particles of different characteristics are urged preferentially towards or away from different DEP regions of the array, so that the particles can be characterised. A flowing fluid is used.

15

The technique of travelling wave DEP is also described by Pethig, chapter 11, pages 93 to 97. One use of the technique is described in WO 97/27933, University of Texas, in which a particle-containing liquid is caused
20 to flow through a flat cell over an array of comb-like electrodes to which signals at different phases are applied so that by a combination of travelling wave DEP, levitation, and field flow fractionation, separation and characterisation of the suspended particles is possible.
25 A flowing fluid is used.

In conventional (i.e. using stationary rather than travelling or rotating fields) DEP, it is also known to use castellated electrodes of the type illustrated in
30 Figure 1, in which each electrode 10 comprises a straight linear backbone 12 having arranged alternately on opposite sides semi-circular protrusions 14.
~~Alternatively, the protrusions can be essentially square~~
in shape. In an electrode array, the protrusions 14 on
35 neighbouring electrodes can be aligned as illustrated, or offset. The electrodes are used for conventional DEP, i.e. for non-travelling fields.

Throughout this specification, the term "particle" is used to include biological cells, bacteria, viruses, parasitic microorganisms, DNA, proteins, biopolymers, non-biological particles, or any other particle which
5 may be suspended in a liquid, in which a dielectrophoretic force can be induced. It also applies to chemical compounds or gases dissolved or suspended in a liquid.

10 According to the invention, a dielectrophoretic cell comprising an array of elongated electrodes, and means to apply at least one electrical signal to the electrodes, in which each electrode has a notional
15 electrode having one or more deflections from the notional central axis, and the electrodes in the array being in register.

In the Shorter Oxford Dictionary, "deflection" is
20 defined as "1. The action of bending down - bent condition; a bend or curve. 2. The action of turning, or state of being turned from a straight line or regular course."

25 In one example, the electrodes are serpentine in shape with their curvatures in register. In another example, the electrodes are zig-zag in shape with their points in register.

30 In one example, the electrodes in an array are all identical and parallel to each other. In another example, the shape of the electrodes alters gradually
along the array.

35 Also according to the invention, a dielectrophoretic method comprising placing a suspension of particles in a liquid in the vicinity of an array of electrodes, the

array being defined and applying at least one electrical signal to the array whereby particles are included in or excluded from regions of the electrodes corresponding to the maximum electrode curvatures. Alternatively,
5 particles may be included in or excluded from regions of the electrodes corresponding to minimum electrode curvatures.

10 The invention will now be described by way of example only with reference to the accompanying drawings in which:

Figure 2 indicates schematically a travelling wave dielectrophoretic (TWD) system;

15 Figures 3A, B, C, D, E, F and G indicate various arrangements of TWD electrodes;

20 Figures 4A, B, C, D, E and F are successive photographs of an experimental separation of particles by TWD;

Figure 5 illustrates an array of TWD electrodes particularly suitable for separating two particles of two or more different types;

25 Figure 6 illustrates schematically an alternative array of TWD electrodes;

30 Figure 7 illustrates an alternative arrangement of serpentine TWD electrodes;

Figure 8 shows a combination of conventional and serpentine TWD electrode arrays;

35 Figures 9A and 9B illustrate respectively an array of electrodes for static dielectrophoresis and appropriate electrical connections for the array; and

Figure 10 shows a variation of Figure 3A.

In Figure 2, a glass substrate 20 has on its upper surface an array 22 of serpentine electrodes, each of which is connected by a multiple connector 24 to a signal generator 26. The substrate 20 can be covered by a protective cover 28 (conveniently a second glass substrate), the substrates being separated by a spacer, not shown, to form a thin cell. A suitable spacer is a plastic strip. In a variation (not illustrated), the electrode array 22 may be fabricated on the protective cover 28.

The DEP cell is illuminated from below by a light source 30, and is viewed from above by an optical microscope/video recorder 32 connected to a display screen 36.

In use, a suspension of particles in a liquid is placed on a substrate 20 and the cover 28 put into place. The signal generator 26 is arranged to apply signals of different phases to the electrodes in the array 22. For example, the signal generator 26 may be a four-phase sinusoidal signal generator, connecting successive electrodes to signals of relative phase 0° , 90° , 180° and 270° , and then repeating the cycle across the whole array 22. As is well-known, such an array generates travelling wave DEP conditions.

The DEP cell is illuminated by the light source 30 and is viewed on the screen 36. In transmission, particles will be seen as distinct areas, and their movement can be clearly seen on the screen.

It is to be noted that there need be no liquid flow through the cell.

Figures 3A to 3F illustrate six different serpentine electrode arrays. In each illustration, the arrows indicate the general directions of travel of the particles under the influence of the travelling wave field, and also indicate the areas of travel within the field.

In Figure 3A, each electrode is sinusoidal in shape. In the Figure, three sinusoidal cycles are shown, the maxima and minima of each sinusoid being in register, i.e. in alignment. The arrows correspond to these cyclical maxima and minima, showing regions in which the particles travel. Three arrows point in one direction with two arrows, intermediate the three, pointing in the opposite direction; the arrows can be regarded as indicating channels of travel, and show that simultaneous travel in opposite directions is possible by different types of particle. The arrangement can be regarded as a traffic control system - particles travelling in opposite directions do not collide.

In known travelling wave DEP arrangements using essentially straight, parallel electrodes, the general travelling wave force is the time averaged translational travelling force which occurs perpendicular to the electrodes.

In the serpentine electrode arrays according to the invention, the general travelling wave force is indicated by the arrows; the force "concentrates" the particles into certain regions and disperses them from other regions depending on electrode shape. Put another way, the particles are included in some regions and excluded from other regions of the travelling field.

Conditions can be selected so that particles of interest travel in one direction, and other particles travel in

the opposite direction.

In Figure 3B, each electrode comprises a series of half sinusoids. All of the particles travelling from left to right in Figure 3B can be regarded as travelling in
5 separate bands in the direction of the arrows.

In Figure 3C, each electrode comprises an elongated "C" shape. All of the particles travelling from left to right in Figure 3C are excluded from the outer regions
10 of the travelling field. This may be beneficial when there is a physical wall, at the edge of the field, thus avoiding bursting or other damage to the particles and preventing loss in the process as particles stick to the
15 adjacent wall. An additional effect is that "clogging" as a result is reduced, i.e. the tendency of multiple particles to stick together in a clump at a wall surface may be minimised.

20 In Figure 3D, each electrode has the form of a single half sinusoid connected between straight side arms. In this array, particles travel in correspondence with the curved part (mainly at maximum curvature). Particles travelling from right to left are excluded from the
25 areas corresponding to the central curved part of the array. The arrangement can be regarded as a one-way channel or a valve.

Figure 3E is similar to Figure 3B, except that each
30 electrode is slightly offset from its neighbours so that the positions of maximum curvature of each electrode are arranged along parallel curves. The four channels
~~indicated by the arrows are curved, so the arrangement~~
can be used to guide particles round corners of smaller
35 radius than previously possible.

Figure 3F is similar to Figure 3A, except that the

electrodes are zig-zag in shape instead of serpentine.

In Figure 3G, the electrodes are straight-line approximations to the sinusoids of Figure 3A, with each
5 full sinusoid being represented by five straight lines; or the electrodes can be regarded as the zig-zags of Figure 3F with flattened points. The arrows in the
channels now point in both possible directions of travel, in contrast to the arrows in Figures 3A and 3F
10 because particles in the straight-line part at the centre of the channels will remain in that region, regardless of the direction of travel. Particles in the 'transition' region of electrodes, i.e. between the channels, will move as illustrated in Figures 3A and 3F.
15 The arrangements of Figures 3A and 3F are therefore preferable.

Inspection of the Figures 3A to 3F will show that a common feature of all the electrodes is that they have
20 two or more different curvatures, either curves of opposite direction, or a curved part and in some cases straight portions. In another example, an electrode array could comprise a series of C-shaped electrodes, i.e. of a single curvature. Other shapes of serpentine
25 electrodes are also possible.

By selection of appropriate shapes of serpentine or zig-zag electrode arrays, it is now therefore possible to guide travelling particles into channels, to form them
30 into bands, to guide particles away from a mechanical constraint on the liquid flow, such as apparatus walls, and to guide them more easily round corners. Particles
can be included in a particular area of the DEP field, or excluded from it. This allows particles to be
35 accurately positioned in a travelling field, thus easing their detection. In addition, the technique can be used to guide particles towards, e.g. an antibody-coated

object or surface.

Figure 4 illustrates particle movement using the electrode array of Figure 3A and the general arrangement of Figure 2. Sixty four sinusoidal electrodes in an array 22 were fabricated on a glass slide 20 by photolithography, and comprise a layer of chromium covered by a layer of gold. Each electrode is approximately 10 micrometers wide and the inter-electrode spacing is about 30 micrometers in the central channel regions. A culture of live yeast cells suspended in water was used, the cell concentration being 10.2 million cells per millilitre, and the conductivity of a suspension being $105\mu\text{s}$ per centimetre. Prior to the experiment, the electrodes were soaked in ultra pure water for over an hour to help to clean them. In the experiment, it was found that at a frequency of 150 kilohertz only levitation of the particles, as a result of a negative DEP force, occurs with no translational component.

In the experiment, a frequency of 150 kilohertz at 3 volts peak to peak was applied to all the electrodes in the array 22 at different phases as explained above. The yeast cell suspension was then applied over the electrodes and a cover slip 28 placed on top. The 150 kilohertz signal caused the particles to levitate above the electrodes and minimises sticking of the yeast cells. After a few seconds, a 50 kilohertz travelling wave DEP field was applied; the yeast cells immediately started to move along the travelling field and started to form into bands as can be seen from Figure 4A.

The large arrows indicate the general direction of movement of the cells, the small arrows indicate local movement of the cells as they are excluded from one channel and included in another, so that the cells form

into bands. At the left hand side of the photographs, five channels are each given a channel number.

Figure 4B is a photograph taken about three seconds
5 after Figure 4A; the cells can be seen to be moving to the right, and are more closely banded in channels 2 and 4, and are largely excluded from channels 1, 3 and 5.

Figure 4C was taken after the travelling wave field
10 direction was changed. The cells are now travelling from right to left, and the bands can be seen moving out of channels 2 and 4 and into channels 3 and 5. Figure 4D was taken three or four seconds later, and the migration into bands is even more marked.

15 Figure 4E shows the bands a few seconds later, and illustrates that the cell movement is beginning to leave a clear area in channels 3 and 5 as the cells are moved to the left.

20 Figure 4F is a photograph taken further along the channels to the left, showing that the bands are uniform along the channels.

25 The small number of cells which are speckled over the electrodes are stuck to the glass and are not moving. At such high cell concentrations, some sticking of the cells commonly occurs; this can be reduced by use of special coatings on the glass, or by using chemical
30 agents such as surfactant or biochemical additives such as proteins (e.g. casein, denatured albumin) and using a polymeric material as a substrate, or placing a film of
~~polymeric material over a glass substrate.~~

35 The experimental results in Figure 4 show that particles can be formed into bands, along the direction of the travelling field and will move in those bands. At high

particle concentrations, travel in bands has been found to be particularly effective. The use of serpentine electrode geometries thus permits very high particle concentrations to be handled more easily than has previously been possible.

If two particle populations are present which are of different properties, conditions can be selected so that they are caused to travel in opposite directions unhindered, allowing separation of the two types of particle. The technique works for low particle concentrations and has been found to be particularly effective when particle concentration is very high, such as millions or tens of millions of particles per cubic centimetre, or even higher. Useful potential commercial applications may be removing bacteria from saliva or stools; removing stem cells, foetal cells or cancer cells from blood; or removing meningital viruses from spinal fluid.

Experiments have been completed with human blood cells using a similar electrode arrangement to those shown in Figures 3A and 4. The electrodes used were $8\mu\text{m}$ wide with $17\mu\text{m}$ inter-electrode spacing in the central channel regions. Experiments were completed at very high cell concentrations with a dilution of 10 times of whole blood, a concentration of approximately 5×10^8 cells per millilitre (I.e. 500 million cells per cubic centimetre). Multi-phased signals were connected to the electrodes and the blood cells moved with TWD forces. A dilution of 20 times whole blood (I.e. a concentration of approximately 2.5×10^8 cells per millilitre) was

found to be preferable where separation rather than just the movement of particles is desired, particularly where there is a large disparity in concentration between the cells to be separated. Separation of white from red blood cells was undertaken at 20 times dilution.

Disparity between cell concentrations was considerable, there being approximately 700 red blood cells for every white cell.

5 In the paper "Electromanipulation and separation of cells using travelling electric fields", J. Phys. D: Appln. Phys, 29, pages 2198-2203 (1996), Talary et al

10 describe the separation of viable and non-viable yeast cells using TWD electrodes. The yeast cells were of the same order of size as blood cells and concentrations of approximately 1×10^4 cells per ml were subjected to TWD forces using conventional electrodes of width $10\mu\text{m}$ and inter-electrode spacing of $10\mu\text{m}$. From the figures included in the publication, it can be appreciated that

15 concentrations of the order 1×10^4 cells per ml represents close to the upper limit for the efficient manipulation and separation of cells using TWD with conventional electrode arrangements. This can be compared to the cell concentration of 2.5×10^8 cells per

20 ml used with serpentine electrodes of the arrangement in Figure 3a, representing an increase of 25,000 times more cells per ml. Furthermore, in the publication by Talary et al, similar ratios of differing cell types (i.e. viable and non-viable yeast) were manipulated, where, as

25 in the manipulation of whole blood cells using serpentine TWD electrodes, the ratio of red blood cells to white blood cells was in the order of 700:1 (a considerably more complex separation). Thus by means of this invention, considerably greater particle

30 concentrations and greater particle type disparities can be handled, and the particles separated.

By means of the arrangement of the invention, low

35 concentration of particles may also be handled and the particles manipulated, characterised and separated with increased levels of control compared to the application

of straight parallel TWD electrodes. The invention may be applied to all ranges of particle concentration to effect, although in application its benefits may be most marked when handling high concentrations.

5

The inventive technique also overcomes a previous disadvantage of DEP in that particles travelling along a

travelling field can tend to drift - the "focussing effect" achieved by the present invention minimises such drifting. Movement under a DEP field can now be distinguished from hydrodynamic fluid flow, which can cause comparatively substantial drifting. Hydrodynamic fluid flow can be induced by heating effects caused by the electric fields.

15

Figure 5 illustrates an electrode arrangement for separation of differing types of particle of different properties and different concentration. Conditions are selected so that the particles respond to the same travelling field by travelling in opposite directions; this can be achieved by changing, e.g. the properties of the applied voltage signals, or the permittivity or electrical conductivity of the suspending liquid, or changing the temperature or even adding a chemical to the suspending liquid.

As will be seen from Figure 5, the electrodes at the upper part of the Figure are in the form of two cycles of a sinusoidal curve in register, but at the lower part of the Figure, the electrodes are in the form of two cycles separated by an almost straight part, the electrode shape gradually changing from one to the other.

35 In operation, the sample suspension can be introduced on to the lower part of the electrode array or placed directly on the whole electrode array. Particles of the

higher concentration are arranged to move towards the top of the Figure in the central channel, while particles of the lower concentration are caused to move downwards along the two outer channels and are caused to
5 diverge away from the central area of the array.

In practice, the particles of high concentration may trap some of the particles of low concentration and carry them upwards. This is opposite to the effect of
10 the travelling wave DEP on the low concentration particles, and eventually they may free themselves and move into the two outer channels as required - a substantial length of the central channel maximises this possibility, for example 0.5 to 5 cm. To further assist
15 this escape from trapping, the travelling wave field may be intermittently switched off, allowing the particles to disperse out of their band a little, and therefore assisting the lower concentration particles to escape. The same "sifting" effect can be achieved by
20 intermittently reversing the field direction. This "sifting" effect is especially useful when working with particles which tend to clot together, such as blood cells.

25 The Figure 5 arrangement may have application, for example, in separating organisms such as salmonella from native E-coli and bacteroids in a sample of stool or separating cancer cells from blood. Cell separation using this sifting effect can also be achieved using
30 other serpentine electrode geometries such as that of Figure 3A or 3B.

When there is a requirement to separate or concentrate or dilute one type or more of particle in a volume of
35 liquid and to discard a second type of particle, the arrangement of Figure 6 can be used. The electrodes are shown as thick lines, straight or curved, but each

electrode is in fact a serpentine electrode such as that illustrated in Figure 3A.

The serpentine electrodes are arranged in two areas; a
5 central area A, in which each serpentine electrode has a
straight axis, and the axes are parallel and transverse
to the Figure - as indicated in the magnified view -

and an outer area B in which the axes of the serpentine
electrodes are "U" shaped. The outer area B therefore
10 has two side arms B1, B2 in which the electrode axes are
straight, and a central connecting part B3 in which the
electrode axes are curved.

The suspension of the mixture of particles is placed in
15 contact with electrodes in both areas A and B, and the
separation takes place in three stages:

1. Signals are applied to the electrodes in central
area A so that particles of a first desired type
20 travel downwards in the Figure and collect at the
lower edge of the central electrode area, and
particles of the second type travel upwards and
move over the outer electrode area B. By selection
of appropriate electrode shape, the particles
25 travel in opposite directions along different
channels.

2. Signals are disconnected from the electrodes in the
central area A, and applied to the outer area B so
30 that particles of the desired type travel inwards
to the inner area A, and particles of the other
type travel outwards and off the edges of area B
and are discarded.

35 3. Signals to area B are disconnected and area A is
reconnected, so that the desired particle type
moves downwards and is collected at the bottom of

the central area.

In a variation, a multi-layer fabrication technique is used, and the inner and outer areas A and B are overlaid at their edges, separated by a thin insulating layer; there is then no area in which particles may become trapped. For increased versatility of particle

manipulation, different regions of electrode areas A and B may be controlled separately.

10

Once the technique of the invention has been applied, the separated or concentrated particle type of choice can be directed to a position at which they can be analysed or characterised by a further DEP analysis, or by any other analysis technique such as optical, ultrasonic, electrical, magnetic, PLR, FISH, etc.

15

In all of the described arrangements, reference has been made to placing the liquid/particle suspension on the DEP electrode array. In a first alternative, the suspension may be placed on a substrate which carries the electrode array on its opposite face. In a second alternative, the suspending liquid may first be placed on or adjacent the electrode array and the particles may be introduced afterwards; for example, in the Figure 5 array, the particles could be introduced in the central area at the bottom of the Figure. In a third alternative, the suspension may be placed between two or more opposing electrode arrays fabricated on separate planar substrates or on a tubular substrate. However, none of arrangements depend on a fluid flow arrangement such as that used in conventional DEP. Fluid flow may be used, but it is not a requirement.

20
25
30

35 A further variation is shown in Figure 7. In all previous examples, the inter-electrode spacing indicated by s on Figure 7 has been constant, but in the

variation, the mark/space ratio w/s (where w is the width of the electrode) increases along the electrode array as shown.

5 Figure 7B gives a side view of the serpentine electrodes 42, and indicates the forces on a particle p . The result of the varying mark/space ratio is that the levitation height of particles above the electrode array, indicated by a line L , increases. The forces on
10 a particle P are shown, i.e. an upward levitation force l (the real part of the travelling wave DEP force), a translational force tw (the imaginary part of the travelling wave DEP force), and gravity g . As the particle moves to the right, the translational force
15 decreases as a result of the increasing mark/space ratio; at the same time the levitation height of the particles increases, which results in a further reduction of the translational force as the particle is further from the electrodes 42. At some point along
20 line L , depending on particle size, the relative components of the dielectrophoretic force, the electric field strength, and electrode geometry, the translational force will become zero, so no further travel occurs. Particles of different properties will
25 therefore travel to different distances and reside in different positions. Particle separation is therefore possible.

In a variation, initial levitation is caused either by
30 applying a static DEP field, or by applying a TWD field at a frequency at which the particles do not experience a translational force.

In a further variation, once particles have been
35 separated into different regions as a result of utilising varied mark/space ratio electrodes, it is then desirable to be able to remove them selectively. Figure

8 shows an array of varied mark/space ratio electrodes 44, in this case conventional linear electrodes, closely adjacent an array of serpentine electrodes 46 of the type shown in Figure 3B (I.e. of constant mark/space ratio) for selective removal of particles. The arrays of electrodes may either be fabricated using multi-layer techniques, or be fabricated on to opposing substrate faces. Utilising these two electrode arrays of different geometry in combination allows particle separation as a result of differing properties, and then selective removal of the separated particles.

For example, particles may be introduced as indicated by the arrow I. Particles of differing properties will travel different distances along the array of electrodes 44, and reside in different positions. These particles may then be removed along channels a through to h by the electrodes 46.

Numerous variants exist, using variations of serpentine, and combinations of serpentine and non-serpentine electrodes. Any of the electrode designs of Figures 3 and 5 may be used, or variants thereof. The choice of electrode geometries will depend on the choice of application. Changing the rate of change of the mark/space ratio of the electrodes can be beneficial depending on which particles are to be separated. For example, a linear or non-linear increase in mark/space ratio can be used. By using these variations, particles with very subtle differences may be separated and selectively removed.

All examples described above with reference to Figures 2 to 8 relate to travelling wave dielectrophoresis, although the electrode arrays may also be used to apply static DEP fields. Referring now to Figure 9, a set of serpentine electrodes suitable for static

dielectrophoresis is shown. The electrodes 48 are "V" shaped and arranged in parallel pairs with the inter-electrode gap E being substantially greater than the inter-pair gap P. Each electrode in a pair projects on one side beyond the other electrode in that pair to facilitate connection to electrical connectors 50, 52 connected to opposite sides of a signal source 54.

Typically the electrodes 48 and connectors 50, 52 will be fabricated on a glass slide by photolithography, with the electrodes 48 being gold electrodes nominally 40 microns thick with an inter-electrode gap E also nominally 40 microns. Inter-pair gap P is nominally 200-1000 μ m. The slide carrying the electrodes will typically be formed into a cell with a spacer and a cover as in Figure 2, the chamber height being between 50 and 300 microns. However, for static dielectrophoresis, as is well known, a flow system must be provided by particle suspension to cause movement as indicated by the arrow in Figure 9A. Such a flow system may be a mechanical system or flow may be caused by the well-known technique of applying an appropriate electrical signal to the electrode array.

If the signal applied to the electrodes 48 is of such a frequency that one type of particle in a suspension flowing through the cell experiences a strong negative DEP force, according to known DEP principles, then such particles will be concentrated towards the regions of maximum curvature of the electrodes 48, while other particles flowing over the electrodes and experiencing a much weaker force, will be relatively unaffected.

Particle enrichment is therefore achieved.

The arrangement of Figure 3A refers to a traffic control system, where particles travelling in opposite directions will travel in the channel regions indicated

by the arrows without colliding. Figures 4A to 4F show electrodes of the arrangement of Figure 3A. These electrodes are of the form of very shallow or flat sinusoids. Alternatively, more pronounced or steep
5 sinusoids may be used as shown in Figure 10. From Figure 10, it is clearly seen that the result of steeper sinusoid electrodes is more defined transition regions,

i.e. the regions between the channels. It is also clearly seen that the inter-electrode gaps are
10 significantly greater in the centre of the channels than they are in the transition regions.

The result of variations in the inter-electrode gaps across the electrode array is regional levitation
15 gradients. In the channel regions, particles will levitate higher, while in the transition regions, they will levitate to a lower height. In the centre of the channels, the particles will levitate the highest, while in the centre of the transition region, they will be at
20 their lowest levitation. The effects of this can be very beneficial for separations.

If a static DEP levitating field is applied, or a travelling DEP field where the translational TWD force
25 for the particles is minimal or zero, the particles will levitate above the electrodes and the substrate. This is beneficial for keeping the particles away from the substrate and minimising particle sticking and clogging. In practice, it is therefore preferred to apply such a
30 field prior to application of the particles. Applying such a field to the electrodes of Figure 10 and placing a solution of particles over them, after a few seconds

it can be seen that particles concentrate in the transition regions between the channels, with the
35 particles moving out of the channel regions due to the regional levitation gradient. Particles feeling stronger levitation forces will move more quickly.

After the particles have concentrated in the transition regions, then applying a TWD field will result in particles which feel a strong TWD translational force moving into and along their respective channels. As a
5 result of the regional levitation gradient, the channels will be predominantly free of particles, allowing particles under strong translational TWD forces to
travel freely along them unhindered, improving separation efficiency.

10

The regional levitation gradient has further application. Particles which feel a weak translational TWD force yet a strong levitation force will still move along the dielectrophoretic cell, but the translational
15 TWD force will be insufficient to overcome the levitation gradient. The particles will thus be restricted to movement within the transition region. This can be used to keep these particles from the fast-moving particles experiencing a strong translational TWD
20 force in the channels. This can be considered as a secondary traffic control system in that not only are particles which are travelling in opposite directions prevented from interfering, but also fast and slow moving particles are segregated from each other.

25 Different electrode geometries can be chosen either to enhance or to minimise this. As a further variation, this region levitation gradient can be used in conjunction with fluid flow. A small amount of fluid flow may be applied in the channel to remove particles
30 which experience very weak or no translational TWD force. The fluid flow may be applied from a source external to the dielectrophoretic cell, or, more

elegantly, a signal may be applied to the TWD electrodes which induces fluid flow, as is known. The result is
35 that a weak fluid flow will have minimal effect on particles which experience strong TWD translational force, while particles feeling very weak or no

translational TWD forces will be moved along the dielectrophoretic cell within the transition regions, thus not disrupting particles moving in the channels. The movement of particles in such a manner with
5 hydrodynamic fluid flow may be undertaken with any of the electrode arrangements, and with or without TWD forces.

When separations are undertaken on a suspension of
10 particles with vastly different concentrations, it is beneficial in aiding separation if conditions can be selected such that the particles are made to travel in opposite directions in a TWD field. In this case, it may be beneficial to modify the electrodes of Figures
15 3A, 3F, 4 and 10. In the figures shown, the channels for particles travelling in opposing directions are of the same width. The width of the channels may be changed to more closely reflect the disparity in concentrations of the particles travelling in them.
20 This will make more efficient use of the electrode arrays in terms of particle movement and separation and may aid in allowing higher concentrations to be handled.

The examples have shown that serpentine or zig-zag
25 electrodes according to the invention may be used with both static and travelling electric fields to both enrich and/or exclude and/or include particles from areas of the electrode array and thus areas or regions of a chamber. This has many applications for
30 characterising, separating, and/or identifying groups of, or individual particles. Both stationary fluid or fluid flow may be used in conjunction with the electrode arrays, as may other external forces be used. Both
35 positive and negative dielectrophoretic forces may be employed with the electrodes. Continuous separation of particles of very high concentrations is possible. By utilising these electrode arrays and predominantly

negative DEP forces, no cell trapping is used, and so relatively small electrode arrays may be used to handle very large particle concentrations and very large volumes, with enrichment of the sample resulting.

CLAIMS

1. A dielectrophoretic (DEP) cell comprising an array of elongated electrodes, and means to apply at least one
5 electrical signal to the electrodes, in which each electrode has a notional central axis along its direction of elongation, the electrode having one or

more deflections from the notional central axis, and the electrodes in the array being in register.
- 10 2. A DEP cell according to Claim 1 in which the electrodes are serpentine in shape.
3. A DEP cell according to Claim 2 in which the
15 serpentine electrodes are sinusoidal in shape.
4. A DEP cell according to Claim 2 in which the serpentine electrodes are half sinusoidal in shape.
- 20 5. A DEP cell according to Claim 2 in which the serpentine electrodes are of elongated "C" shape.
6. A DEP cell according to Claim 2 in which the serpentine electrodes are single half sinusoids
25 connected between straight side arms.
7. A DEP cell according to Claim 1 in which the electrodes are zig-zag in shape.
- 30 8. A DEP cell according to Claim 1 in which the electrodes are straight line approximations to sinusoids.
- 9. A DEP cell according to any one of Claims 2, 3, 7
35 or 8 in which the curvature of the deflections from the notional central axis on one side is different from the curvature of the deflections on the other side, whereby

particle transport channels of different width are provided.

10. A DEP cell according to any preceding Claim in
5 which the positions of maximum curvature of each
electrode are arranged in linear alignment.

11. A DEP cell according to any one of Claims 1 to 8
in which the positions of maximum curvature of each
10 electrode are arranged in non-linear alignment.

12. A DEP cell according to Claim 11 in which the
positions of maximum curvature of each electrode are
arranged along a curve.

15 13. A DEP cell according to Claim 12 in which the
electrodes are serpentine and each comprises two
sinusoids, and the positions of maximum curvature of the
sinusoids are arranged along divergent curves.

20 14. A DEP cell according to any one of Claims 1 to 4
comprising a first central array of sinusoidal or half
sinusoidal electrodes, the axes of the electrodes being
straight and parallel, and a second outer array of
25 sinusoidal or half sinusoidal electrodes, the axes of
the electrodes being in the form of nested "U" shapes,
there being provided means to apply electrical signals
of different phases independently to the first and
second arrays.

30 15. A DEP cell according to any one of Claims 1 to 12
in which the inter-electrode spacing in the array varies
along the array.

35 16. A DEP cell according to any one of Claims 1 to 12
in which the electrodes are arranged in pairs with the
inter-electrode spacing being substantially greater than

the inter-pair spacing.

17. A dielectrophoretic system comprising a DEP cell according to any one of Claims 1 to 15, at least a part
5 of the cell being formed of transparent material; means to illuminate the cell; and means to receive illumination transmitted through or reflected from the cell.

10 18. A dielectrophoretic method comprising placing a suspension of particles in a liquid in the vicinity of an array of elongated electrodes in which each electrode has a notional central axis along its direction of elongation, the electrode having one or more deflections
15 from the notional central axis, and applying at least one electrical signal to the array whereby particles are included in or excluded from regions of the electrodes corresponding to the maximum or minimum electrode curvatures.

20 19. A method according to Claim 18 in which the frequency of the electrical signals is selected to cause a negative dielectrophoretic response in a selected particle type in the suspension and there is further
25 provided means to cause the liquid suspension to flow across the electrode array.

20. A method according to Claim 19 in which said means to cause the liquid suspension to flow is an electrical
30 signal applied to the electrode array.

21. A method according to Claim 18 in which electrical
~~signals at different phases are applied to the~~
electrodes, whereby a travelling wave electric field is
35 generated which induces a travelling wave DEP force on said particles, the real part of said force levitating the particles, and the imaginary part thereof causing

the particles to move into certain regions of the travelling field.

22. A method according to Claim 21 further comprising
5 the initial step of applying to the electrode array an electrical signal whereby a static DEP field is generated so as to cause initial levitation of the particles.

10 23. A method according to Claim 21 further comprising the initial step of applying to the electrode array an electrical signal whereby a travelling wave electric field is generated at a frequency such that the particles are initially levitated but experience no
15 translational force.

24. A method according to Claim 18 in which the suspension comprises a suspension of first and second
20 types of particles, the concentrations of the types of particles differing by a factor of at least 1000, and in which the shape of the array of electrodes is selected so that the types of particles are separated.

25 25. A method according to Claim 24 in which the shape of the array of electrodes is selected to prevent particles from contacting a mechanical constraint on the liquid flow.

30 26. A method according to Claim 18 in which the concentration of the suspension of particles is greater than 10^8 cells per millilitre.

27. A travelling wave dielectrophoretic cell
35 substantially as hereinbefore described with reference to any one of Figures 2, 3, 5, 6, 7, 8, 9 or 10 of the accompanying drawings.

ABSTRACT

DIELECTROPHORETIC APPARATUS & METHOD

5 In a dielectrophoretic cell having an array of
electrodes and means to apply electrical signals to the
electrodes, the electrodes comprise a planar array of
serpentine or zig-zag electrodes with their curvatures
in register. The serpentine electrodes may be
10 sinusoidal, half sinusoidal, or elongated "C" in shape;
the positions of maximum curvature of each serpentine or
zig-zag electrode may be arranged in linear alignment,
or along a curve. The cell may be used for static or
travelling wave DEP. Particles travelling in opposite
15 directions in a TWD can do so without interference,
allowing "traffic control". Particles can be
characterised and separated, and particles at high
concentrations, or particles of different types, can be
handled.

20

Fig. 2

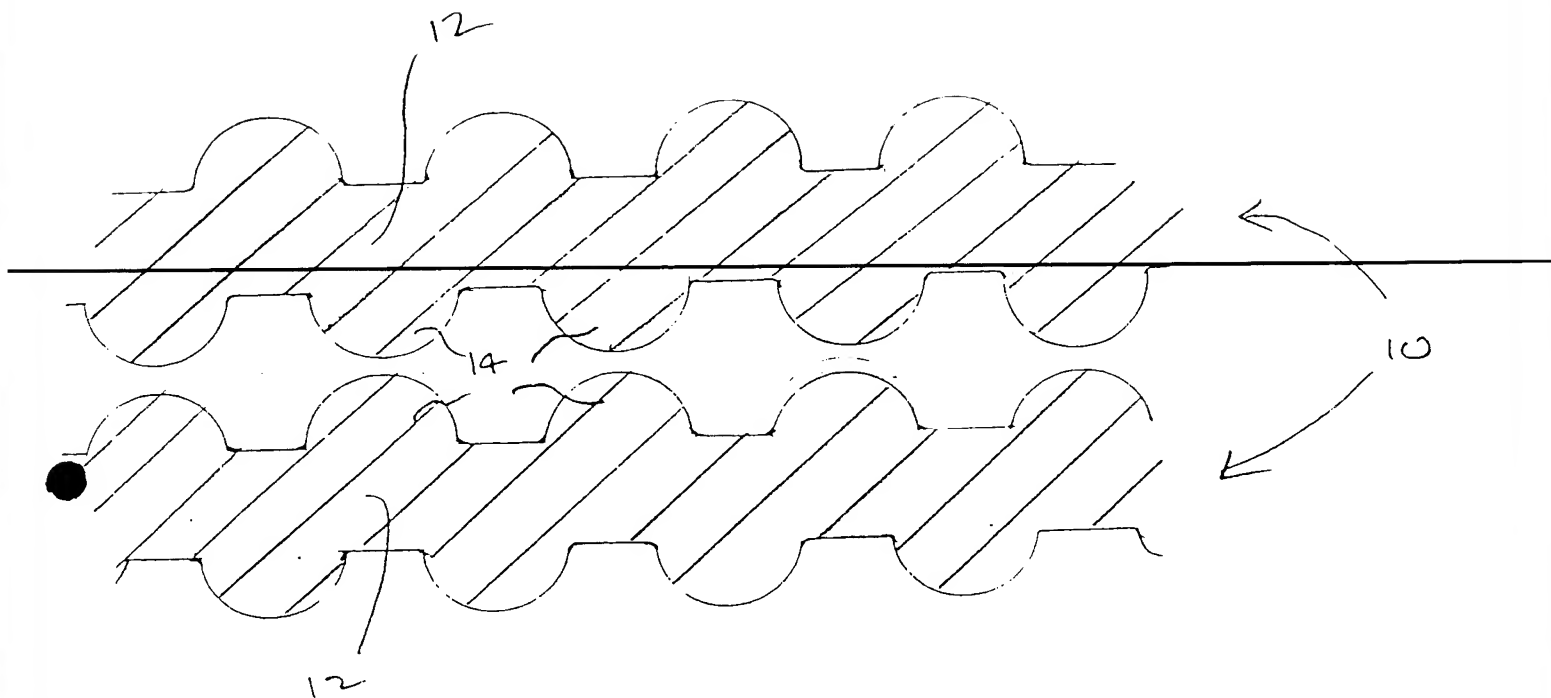


FIG. 1

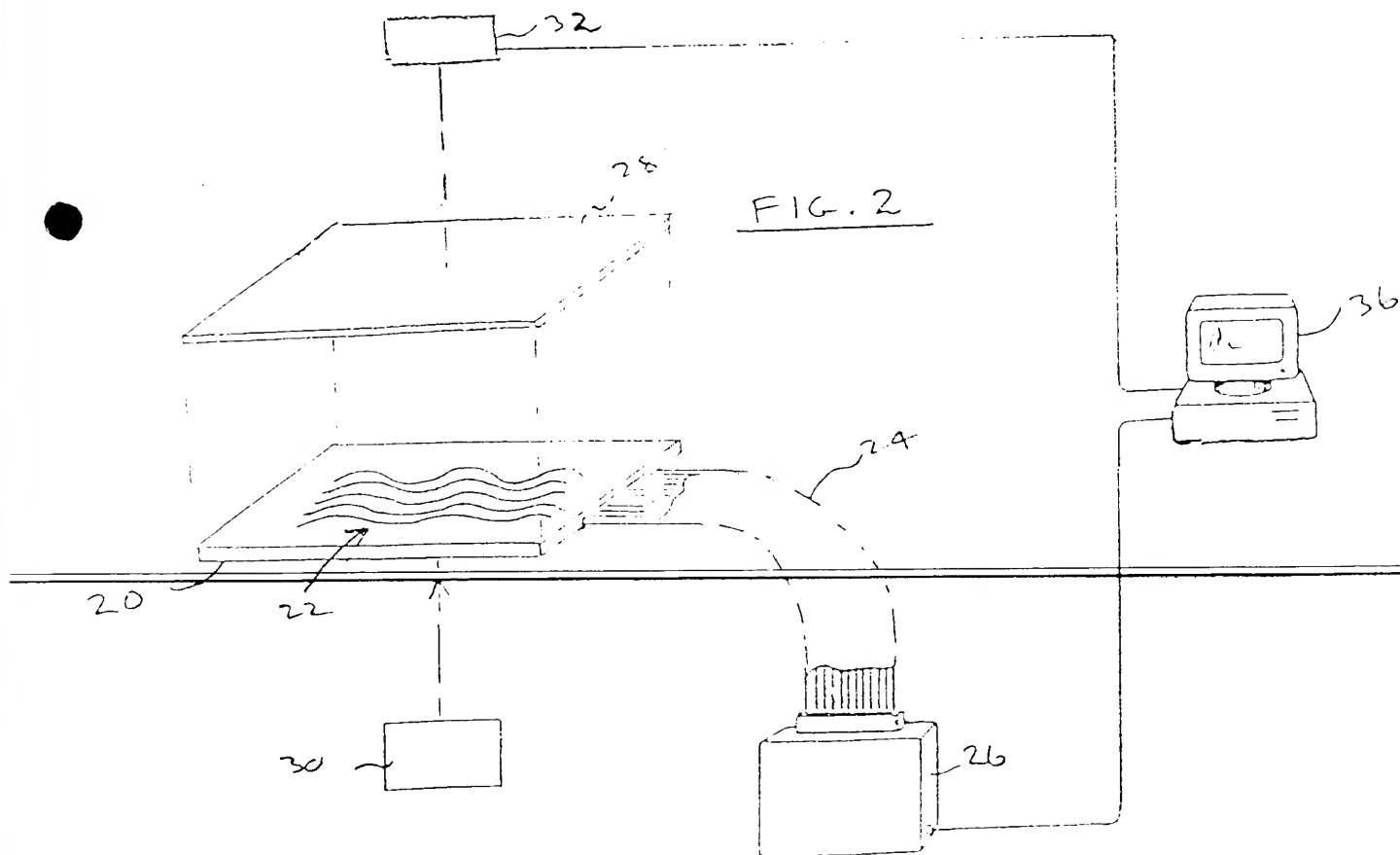


FIG. 2



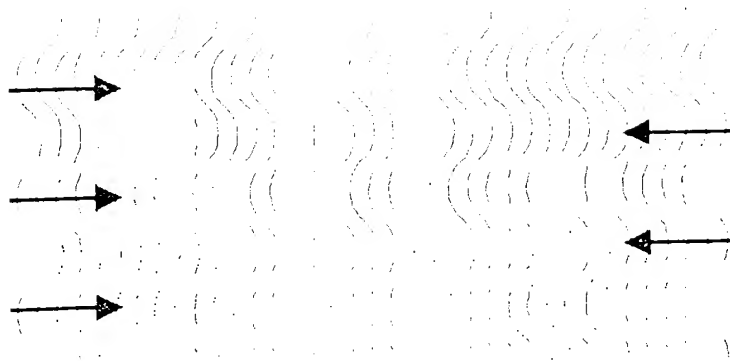


Fig. 3a

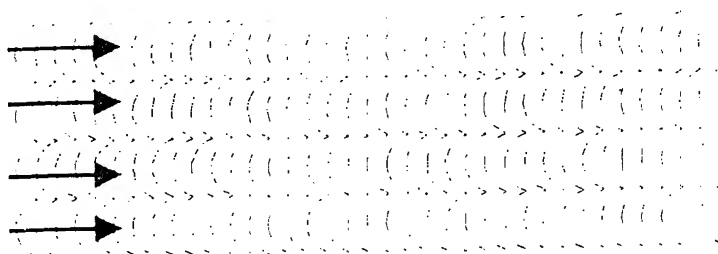


Fig. 3b

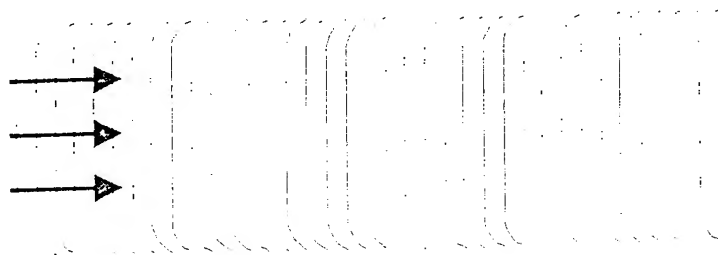


Fig. 3c

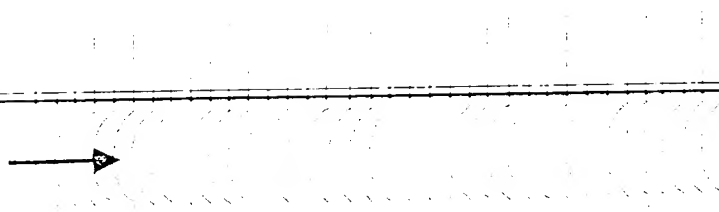


Fig. 3d





Fig. 3e

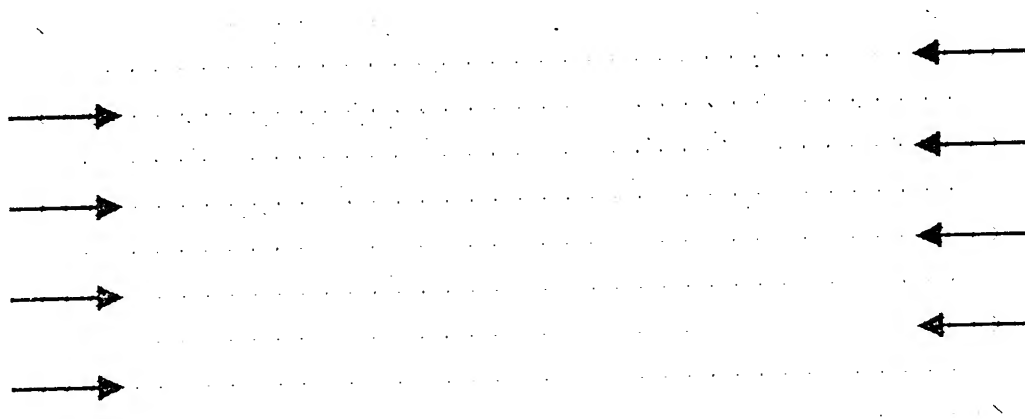


Fig. 3f

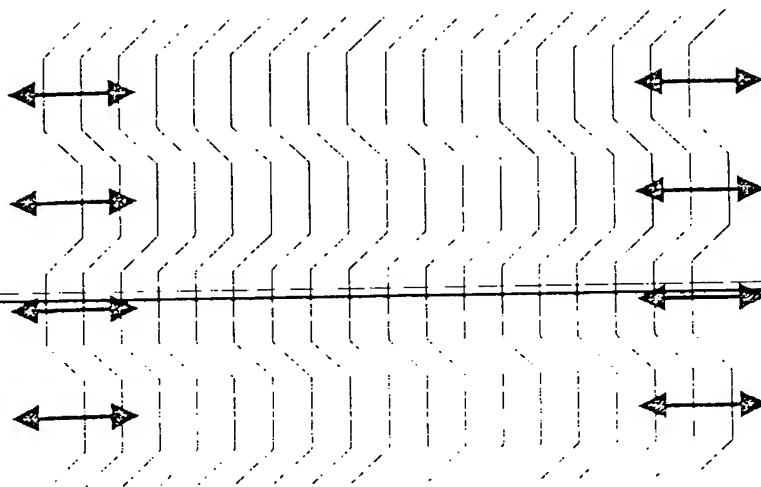


Fig. 3g



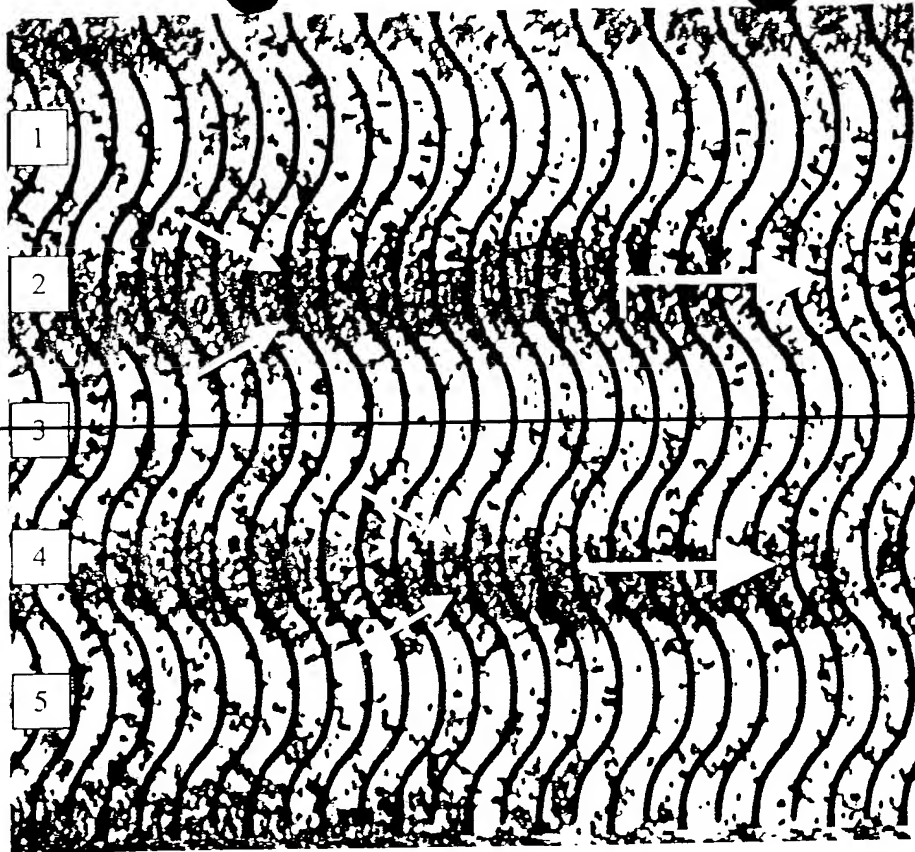


Fig. 4a

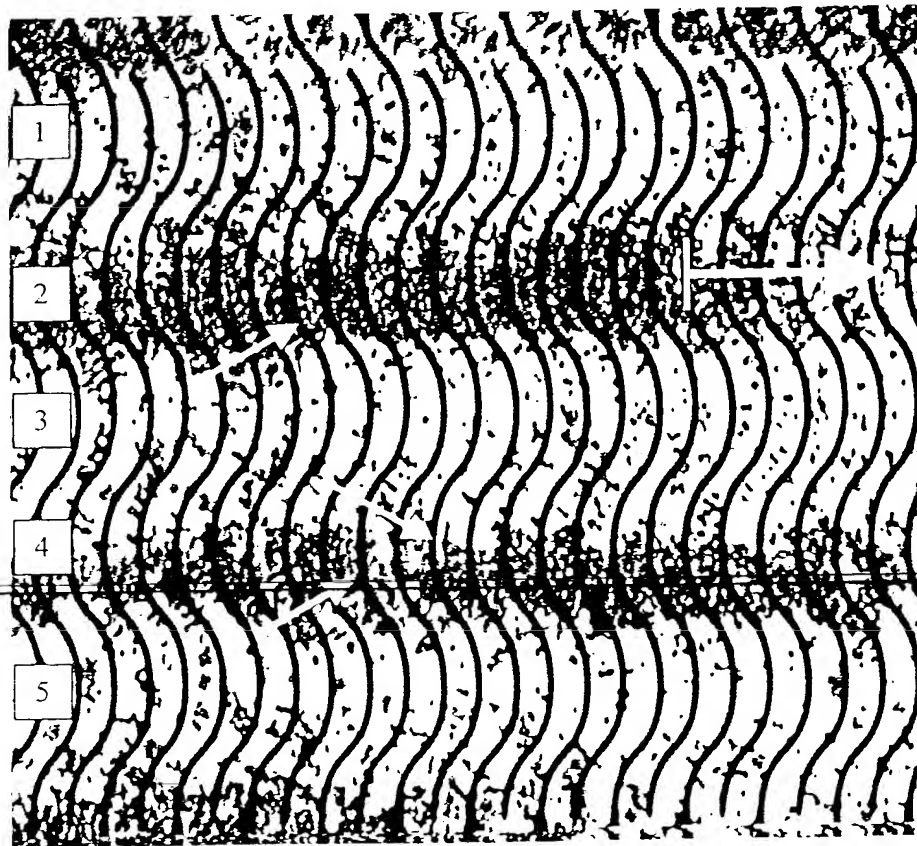


Fig. 4b



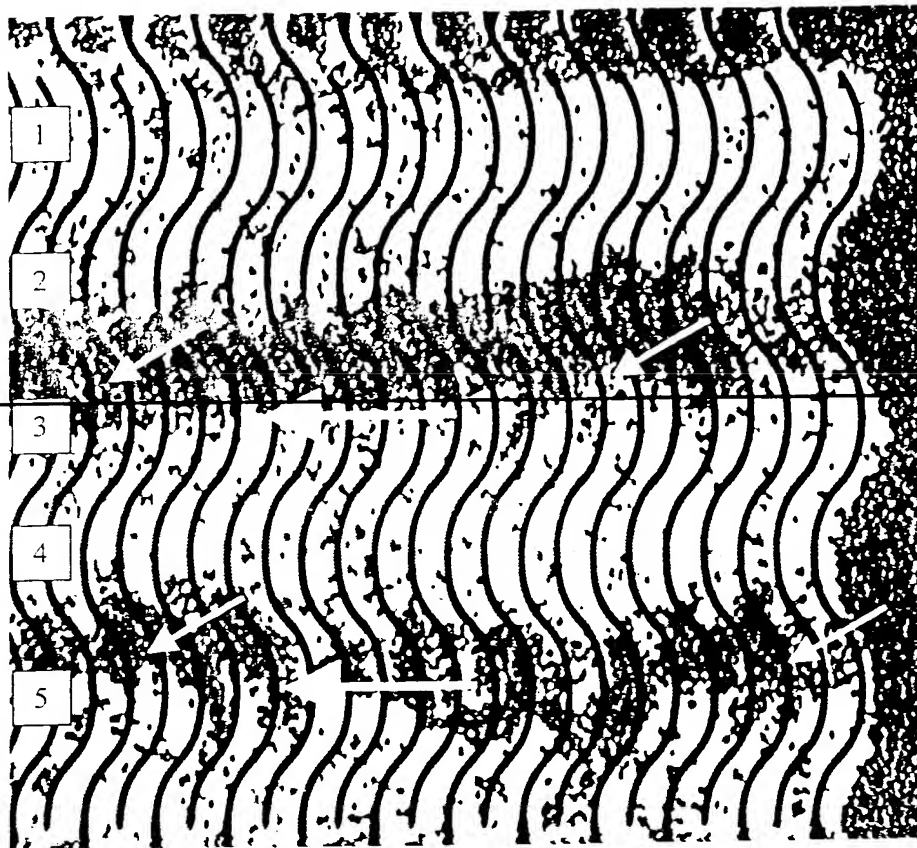


Fig. 4c

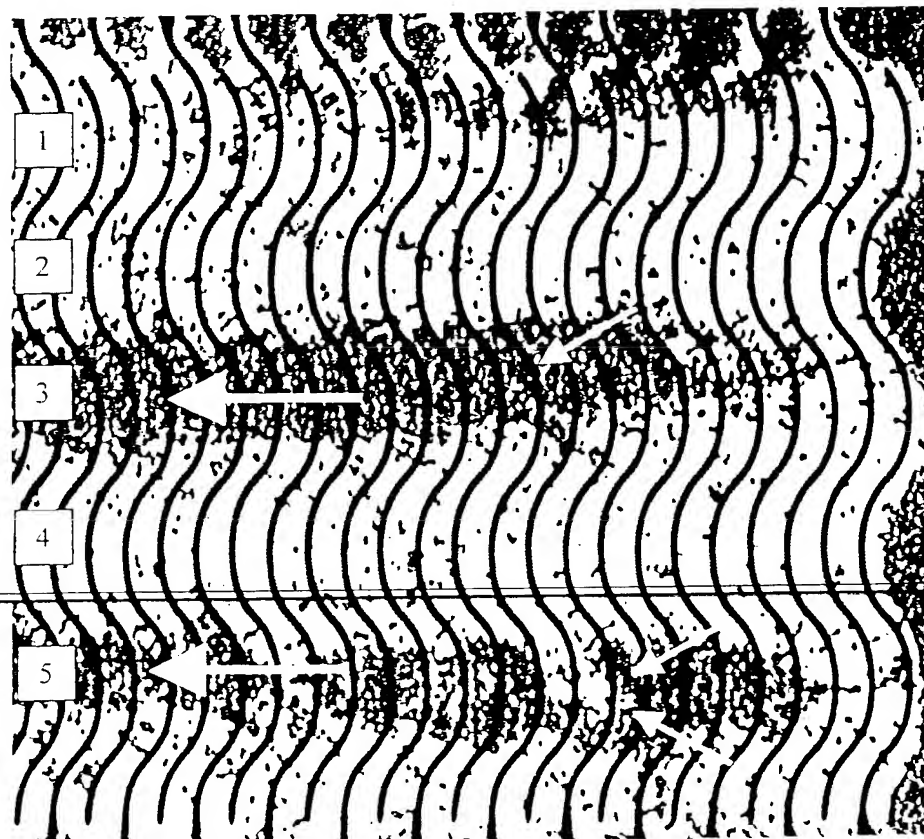


Fig. 4d



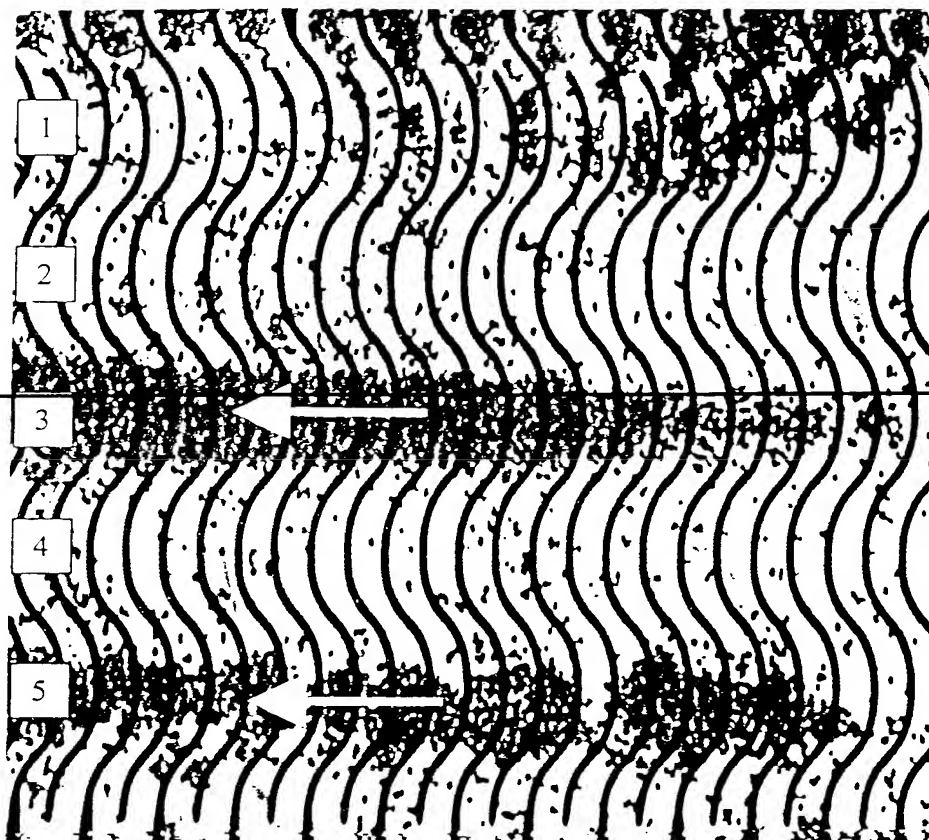


Fig. 4e

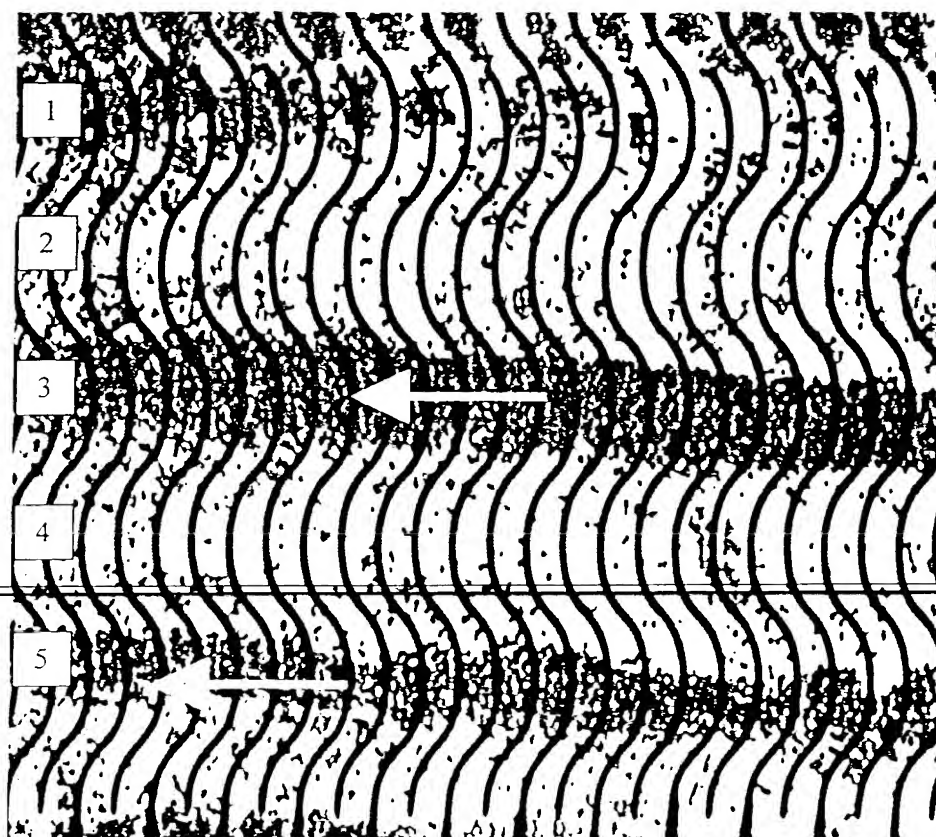


Fig. 4f



7/10

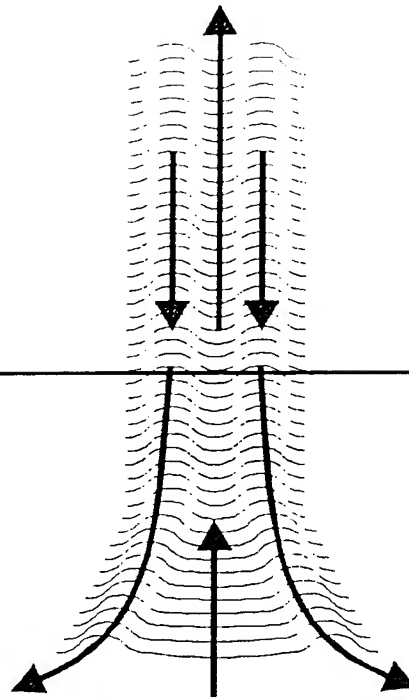


Fig. 5

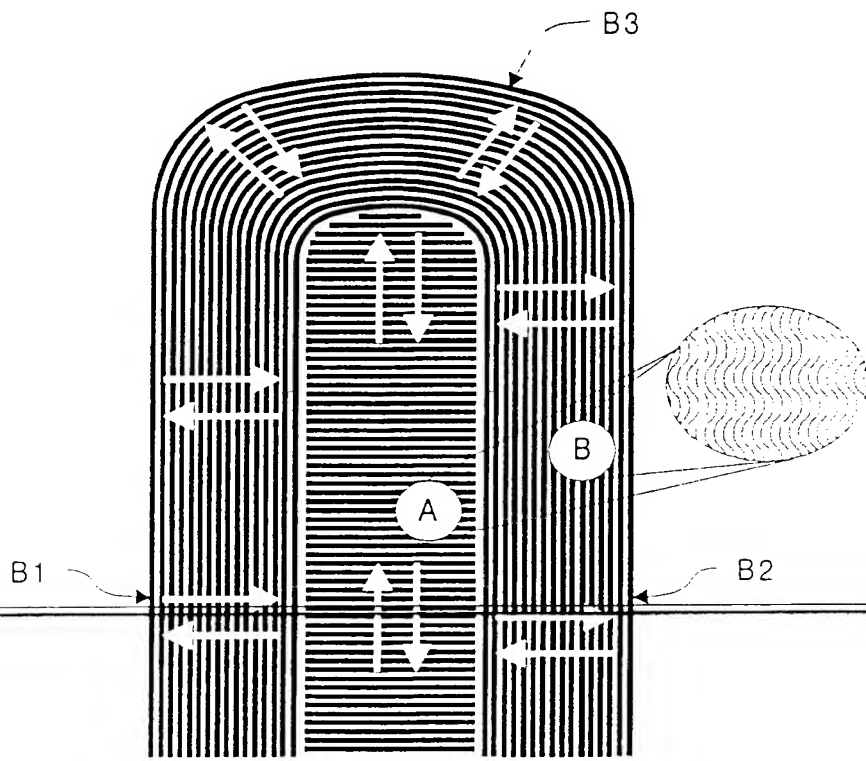


Fig. 6



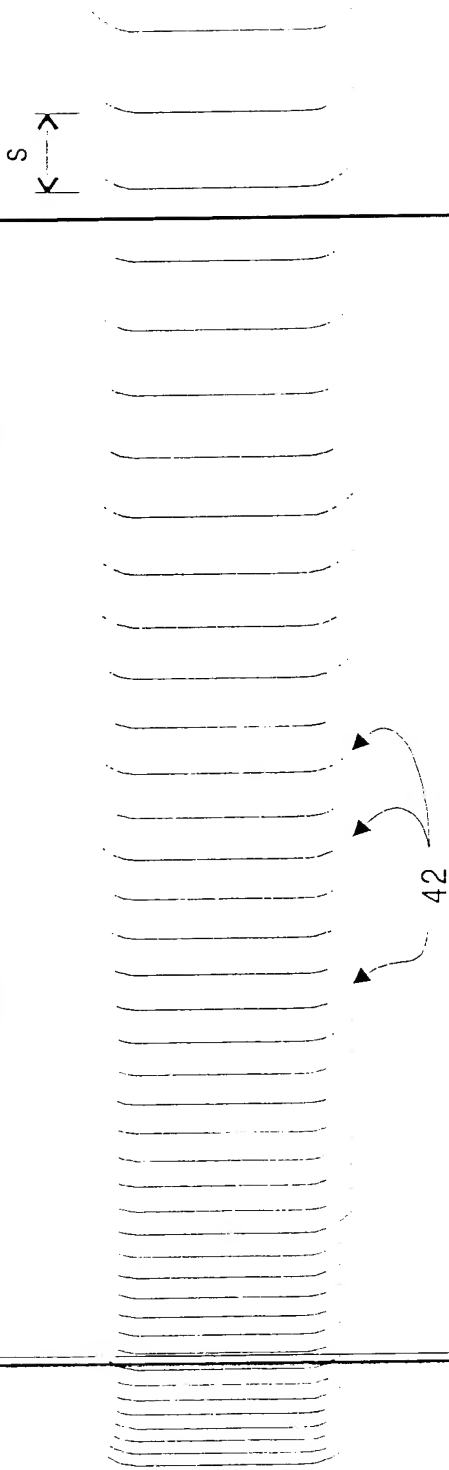


Fig. 7a

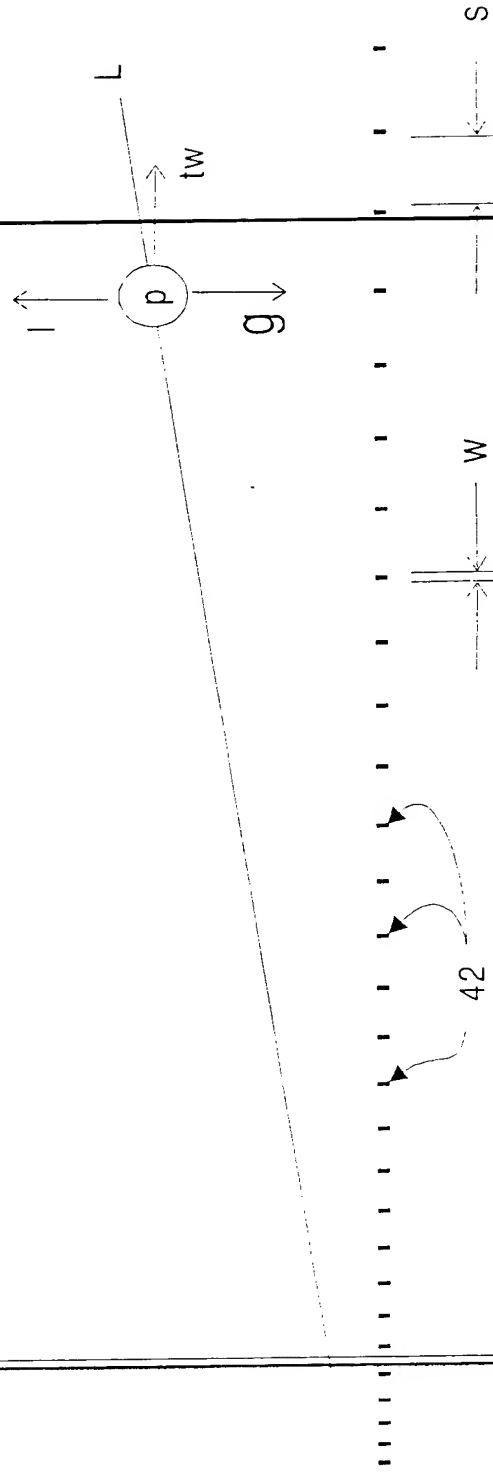


Fig. 7b



9/110

Drive

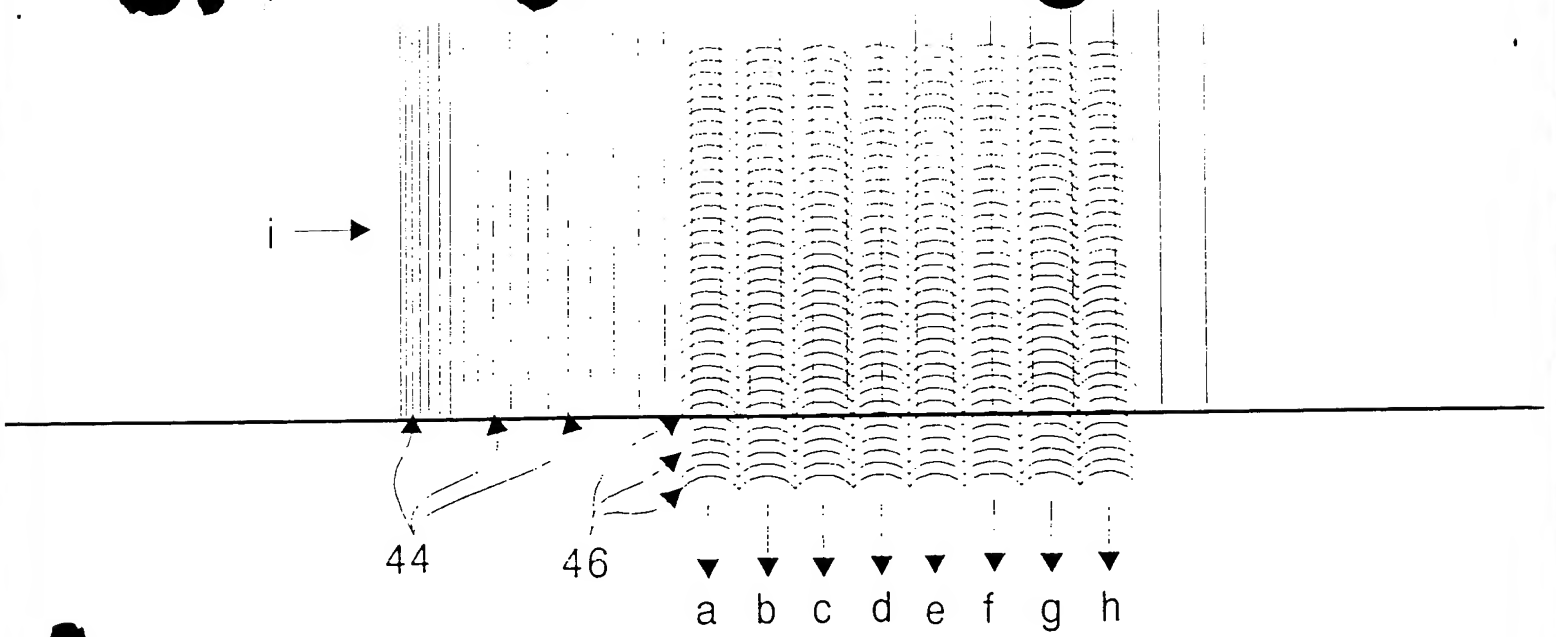


Fig. 8

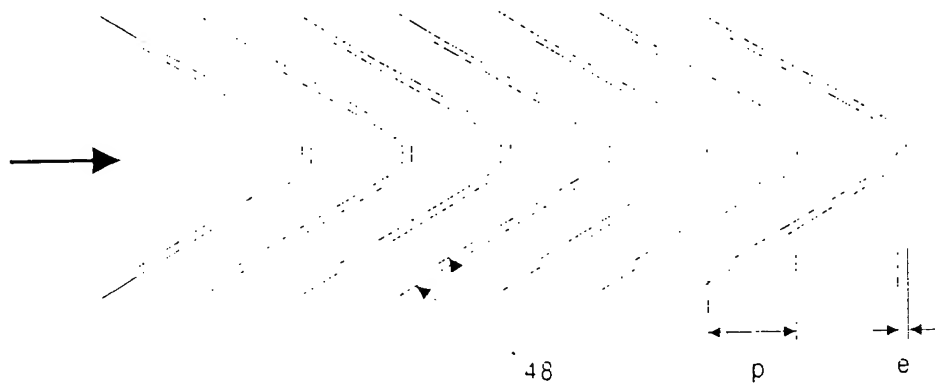


Fig. 9a

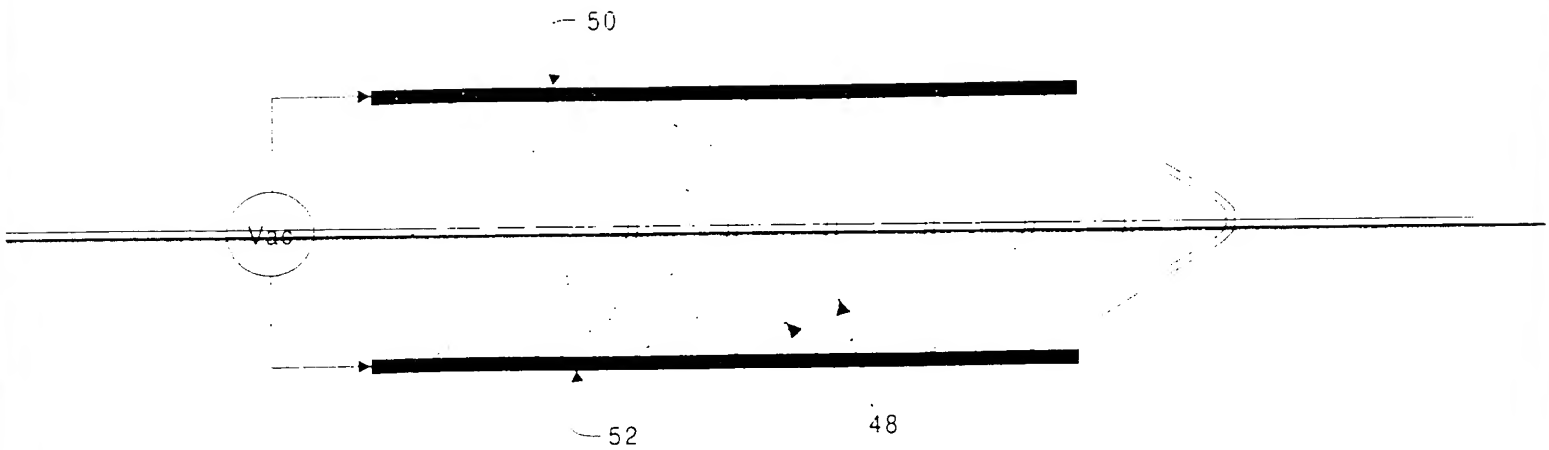


Fig. 9b



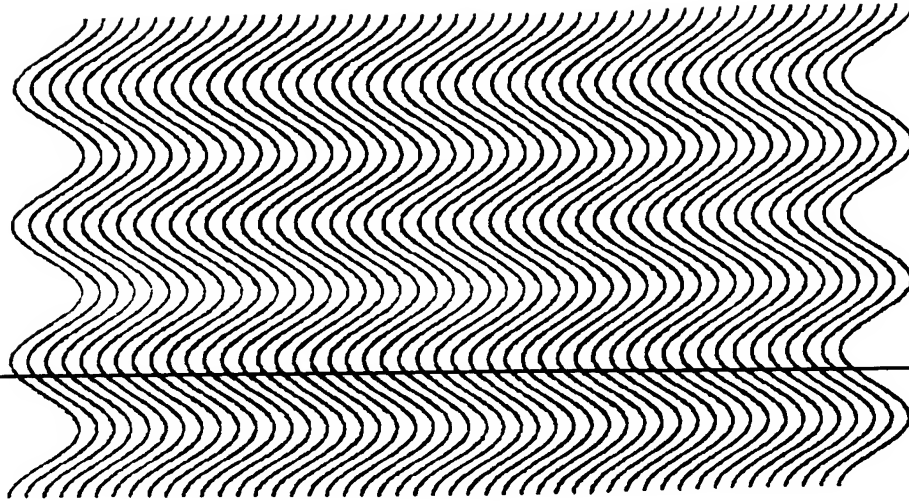


Figure 10

